Serum Selenium in Institutionalized Elderly Subjects and Relation to Other Nutritional Markers

To the Editor:
Nowadays, there is growing interest in the possible relationship between selenium metabolism and the aging process [1]. Epidemiological studies show that selenium may have a preventive role in some degenerative diseases such as hepatic cirrhosis, cardiovascular diseases, and some types of cancer [2, 3]. However, information on the influence of selenium on aging is contradictory, apart from the fact that the process is not yet well understood.

We determined the serum concentrations of Se in 93 institutionalized elderly people in Granada (Spain) as a short-term indicator of human selenium status and its correlation with nutrient intake. In addition, taking into account the role of Se in oxidation, in metabolic changes in plasma lipids, and as a component of glutathione peroxidase, we examined other biochemical markers, e.g., total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides. We likewise studied leukocyte numbers in blood, as an indicator of risk of disease through infection, and established their correlation with serum Se concentrations.

To quantify serum selenium, we used the hydride generation atomic absorption spectrometry technique previously optimized [4]. Daily dietary intake was determined by the 7-day weighed food record, including a day off [5]. Unfortunately, determination of Se intakes was not possible because the software used to calculate the nutritional composition of the foodstuffs did not include Se.

The concentration of serum selenium in the institutionalized elderly people is shown in Table 1. No significant differences according to sex were noted. Depending on the age of the subjects in the two groups considered, the serum selenium decreased significantly in women (P < 0.05) but not in men (probably because of the low number of men examined in the study).

According to Campbell et al. [6], aging per se has very little effect on the status of selenium; that is, intercurrent illness and reduction in food intake are the most important factors in the reduction in the status of this element in old age. In a study comparing healthy elderly subjects (both institutionalized and noninstitutionalized) with a group of young adults, no statistically significant differences between the groups were found in selenium concentrations in plasma or in glutathione peroxidase activity [1]. The same was observed in this study, comparing serum selenium concentrations in institutionalized elderly subjects in Granada (Table 1) with those in younger adult subjects from coastal and mountain towns of the same province [4] (i.e., 74.9 ± 27.3 μg/L selenium). This result could be explained by the known influence of geographical origin on selenium concentrations in the food produced in the area and, ultimately, on the daily dietary intake of selenium [7]; this, in turn, affects the concentrations of this element in the subjects’ serum.

Several studies have found that Se concentrations in plasma [2, 6], and serum [8] of healthy elderly people were significantly lower than those in young adults. However, other researchers investigating people of ages >60 years have indicated that age does not affect the concentration of serum Se [9]. Nonetheless, with regard to the age of the institutionalized elderly people, the concentration of selenium was significantly lower (P < 0.05) in women older than 80 years. This result may be related to the highly heterogenic characteristics of the very elderly, along with a significant decrease in energy intake (from 1850 ± 254 kcal daily in women of <80 years to 1679±234 kcal daily in women of ≥80 years; P < 0.01).

We also correlated serum selenium concentrations with macronutrient intake. The only positive correlation (P < 0.05) was with the intakes of polyunsaturated fatty acids, both for all samples (r = 0.2627) and for samples from women only (r = 0.3637), as was previously found in serum [10] of healthy subjects. This finding could reflect the known positive influence of polyunsaturated fatty acids on selenium bioavailability [8].

Significant relationships (P < 0.05) were observed between the serum selenium concentrations and plasma total cholesterol (r = 0.2965 in all subjects; r = 0.3439 in women) as well as LDL-cholesterol (r = 0.2765 in all subjects; r = 0.3020 in women). The same statistical association between serum selenium and total cholesterol has been previously indicated [11]. The present study thus reinforces the important role of Se in prevention of cardiovascular disease [12].

Finally, we also found a statistically significant negative correlation (P < 0.05) between serum selenium concentrations in institutionalized elderly subjects and numbers of leukocytes in blood in all the subjects (r = −0.2316) and in women (r = −0.1727). However, we saw no statistical difference for the leukocytes in all subjects by sex or age groups. Therefore, the results obtained in the

Table 1. Mean serum selenium in institutionalized elderly subjects by sex and age.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Se concn, μg/L</th>
<th>Total</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>93</td>
<td>63–97</td>
<td>76.02 ± 20.54</td>
<td>21.15–137.90</td>
</tr>
<tr>
<td>Men</td>
<td>24</td>
<td>63–93</td>
<td>77.15 ± 20.56</td>
<td>27.64–105.52</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>&lt;80</td>
<td>77.38 ± 19.11</td>
<td>62.54–101.01</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>≥80</td>
<td>76.60 ± 21.01</td>
<td>66.00–121.00</td>
</tr>
<tr>
<td>Women</td>
<td>69</td>
<td>63–97</td>
<td>75.73 ± 20.54</td>
<td>21.15–137.90</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>&lt;80</td>
<td>83.67 ± 19.48</td>
<td>70.79± 21.29</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>≥80</td>
<td>70.79 ± 21.29a</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from younger age group (P < 0.05).
present study lead us to suggest that this probably reflects the role of Se in leukocyte synthesis and activity, although the mechanism and manner in which Se concentrations regulate blood leukocytes are unclear and require further research. Nevertheless, this result establishes the importance of the conservation of an appropriate status in the elderly, to avoid a reduction of the protective action of Se, and in what way the influence is progressively determine at what concentration of Se is necessary.

We did not observe a clear correlation in the biochemical markers under consideration (serum selenium, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and leukocytes) between the two age groups (<80 years; ≥80 years) and so could establish no relation between serum Se concentrations and cellular aging in the institutionalized elderly subjects under consideration. The fact that most of the experimental studies on influences of Se status in aging have not been able to decisively determine at what concentration and in what way the influence is established in the degenerative diseases associated with old age suggests that more research is needed in this area.

References


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More on Renin

To the Editor:

In an editorial comment [1] on our paper [2], Sealey and Laragh erroneously conclude that the IRMA is a step backward from the traditional enzyme-kinetic plasma renin activity (PRA) assay, and that the new IRMA is not suitable for measuring renin because it comes measure 1–2% of prorenin.

They regard the traditional PRA assay as an accurate measure of the in vivo production of angiotensin (Ang II) the physiologically relevant end product of the renin–angiotensin cascade. In fact, the PRA assay measures the concentration of Ang I that is generated in plasma in vitro after a long incubation period (up to 18 h) under artificial conditions. The PRA assay is an indirect assay of renin and is complicated by the fact that Ang I is usually converted to Ang II and degraded into smaller inactive peptides. Ang I-to-II conversion and Ang I degradation are prevented by lowering the pH of plasma and by adding peptidase inhibitors before the incubation step. Whether this is 100% successful, particularly with such long incubation times, has not been formally tested. These difficulties are not encountered in the direct assay of renin by IRMA.

The prorenin concentration in plasma is higher than the renin concentration, and comeasurement of even a small percentage of prorenin may therefore lead to a sizable overestimation of renin. The important question is: What is the true magnitude of this problem? Our study was designed to address precisely this question, and the answer is simple: The problem is not important enough to render the IRMA unsuitable for clinical use. In contrast to what is stated in the editorial comment, our study demonstrates that the the new assay can readily distinguish low-, normal-, and high-renin hypertension. There was good agreement with the enzyme-kinetic assay not only in the normal- and high-renin ranges but also in the low-renin range, where overestimation would be the most troublesome. Our study also specifically addressed the possibility, suggested in the editorial comment, that comeasurement of prorenin may lead to unacceptably high variability of IRMA results. Between-patient variability in the low-, normal-, and high-renin subjects was similar for IRMA and the enzyme-kinetic assay.

Figure 1 (left) compares the results of the PRA assay according to the method of Sealey [3], obtained after 3 h of incubation, with the results of the same assay after 18 h of incubation in plasma from 16 hypertensive patients with PRA concentrations within the low to normal-range of Sealey’s method. The 18-h results were 39% (20–59%) [mean (range)], lower than the 3-h results. To determine the recovery of Ang I in these
In conclusion, we find no compelling theoretical arguments and no experimental data to support the statement that the new IRMA of renin is a decline in methodology. On the contrary, not only is the IRMA accurate, it also has the important advantage that results obtained in one clinical laboratory can be readily compared with those of other laboratories, because the IRMA results are expressed in units of the internationally accepted renin standard. Results of PRA assays cannot be expressed in an absolute concentration, whereas the amount of renin generated during the incubation time is determined not only by the plasma concentration of renin but also by the plasma concentration of angiotensinogen. We agree with the complaints of Robertson and Nichols [5] that this has restricted the exchange of quantitative information among workers and has seriously stunted scientific progress in the field.

References

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The authors of the editorial referred to above reply:

Drs. Derkx and Schalekamp must excuse us for not putting out the welcome mat for their new renin IRMA [1, 2]. Renin methodology currently available is simple and precise [3]. There is no reason to take a step backward to an assay that at the same time that it measures renin also detects prorenin, a fluctuating component that is always much higher than renin in plasma.

In their enthusiasm for their new tool, Derkx and Schalekamp have lost sight of the fact that determining a subnormal renin concentration in the individual patient is as important as identifying an increased one, especially for hypertensive patients and now also for normotensive patients prone to myocardial infarction or heart failure. Hypertensive patients with low renin concentrations are unlikely to have a myocardial infarction, whereas the amount of renin among those with medium or high renin values is directly related to the subsequent heart attack rate [4]. Studies of >100 000 coronary unit patients [5, 6] show that the heart attack rate is promptly and persistently reduced by specific antirenin system drug therapies. This drug-related benefit involves subjects with medium or high renin concentrations and is not apparent in those with low renin [7]. This relation between plasma renin concentration and cardiovascular pathology is also supported by previous demonstrations in hypertensive animal models of striking protection from cardiac, cerebral, and renal vascular injury after
Fig. 1. Plasma renin enzymatic assay.

There was no difference in the hourly rate of Ang I generation when samples were incubated at 37°C for 3 or 18 h, indicating complete angiotensinase inhibition in the presence of EDTA and a serine protease inhibitor in plasma samples incubated at pH 5.7. Reprinted from ref. 10 with permission.

administration of a specific Ang II antagonist [8, 9].

As we previously pointed out [1], it is inopportune to introduce and proselytize a renin assay [2] that cannot with any certainty identify and discriminate the individual patient with subnormal renin concentrations. The many hypertensive patients with low renin concentrations are at considerable risk of being falsely classified by the proposed new assay as either medium (normal) or high renin. For example, three patients with the same low plasma renin (0.5) but with different plasma prorenins (5, 20, or 200) would be reported as having plasma renins of 0.6, 0.9, and 4.5, respectively. Thus two of the three would be erroneously classified as medium and high renin, although all three have low renin. These wrong results are likely to mislead the physician in assessing cardiovascular risk and deciding on the urgency and mode of treatment. This risk of failure to identify the low-renin patient is unnecessary.

On another point, given Derkx and Schalekamp’s expertise in the field, it is most surprising that they are unable to repeat our work, first published in 1975 and repeated many times since, showing that the 18-h Ang I generation step of the traditional PRA assay is accurate when carried out as described by us (Fig. 1) [10]. However, it is noteworthy that their apparent underestimation of PRA with the 18-h incubation was consistent across PRA concentrations (left side of their figure), whereas the IRMA overestimation was much greater in the lower renin range (right side of their figure). Derkx et al. still do not understand that the reason for the 18-h incubation is not because the Ang I RIA is not sensitive enough (it is sensitive to at least 0.3 ng/mL per hour for a 3-h incubation). Incubation for the sample with low renin is prolonged because the Ang I RIA is not sensitive enough (it is sensitive to at least 0.3 ng/mL per hour for a 3-h incubation). Derkx et al. still do not understand that the reason for the 18-h incubation is not because the Ang I RIA is not sensitive enough (it is sensitive to at least 0.3 ng/mL per hour for a 3-h incubation). Incubation for the sample with low renin is prolonged because the Ang I RIA is not sensitive enough (it is sensitive to at least 0.3 ng/mL per hour for a 3-h incubation). This renin “standard” to which they refer was prepared long ago before modern purification techniques were available. This renin “standard” is not pure, nor was it ever claimed to be pure. When a pure standard of human renin becomes available that does not contain potentially cross-reacting substances, restandardization of direct renin assays will probably be necessary. Meanwhile a pure Ang I standard for the enzymatic renin assay is readily available to all from the National Bureau of Standards.

In any case, these issues are not nearly as germane as is the variable but consistently falsely high renin concentrations detected by their IRMA because of the large and variable prorenin intrusion, which we find wholly unacceptable for characterization of plasma renin concentrations not only of hypertensive patients but also of those with diabetes, hypertension, myocardial infarction, or heart failure. Altogether, therefore, this test does not improve on the presently available approach. If the proposed assay was entirely without cross-reactivity for prorenin, it could perhaps be used successfully to assay renin directly and to explore those rare esoteric situations in which the concentration of renin per se needs to be measured. However, to study disease states in which angiotensin formation is the critical issue, the PRA assay remains the gold standard because it incorporates the concurrent contribution of renin substrate to angiotensin formation and therefore is the most meaningful physiological and pathophysiological indicator of the activity of the renin–angiotensin system.

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References

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Heparin Interference with Sodium and Albumin Assays

To the Editor:
We observed an unusual set of plasma electrolyte and protein values in a sample drawn just before hemodialysis from a 66-year-old man in the outpatient dialysis center. Electrolyte values, determined with the Hitachi 747 analyzer (Boehringer Mannheim, Indianapolis, IN), were within normal limits except for a Na value of 165 mmol/L. When electrolyte values were reassayed with an Ektachem 750 analyzer (Johnson and Johnson, Rochester, NY), all values agreed except for Na, which was 152 mmol/L. Other unusual values from the Hitachi 747 included an albumin of 58 g/L and a total protein of 55 g/L. Ultracentrifugation (because the sample was mildly lipemic) did not change these results. Plasma electrophoresis revealed an ordinary pattern of globulins and albumin.

To determine whether sodium heparin was responsible for this perplexing set of values, we diluted routine patients’ serum with 0-300 mL/L of a sodium heparin solution (5000 USP units/mL; Elkins-Sinn, Cherry Hill, NJ). Sodium values from the Ektachem instrument for samples containing 100-300 mL/L sodium heparin solution were consistently lower than those from the Hitachi, and the samples containing 200-300 mL/L heparin solution (final heparin concentration 1000-1500 units/mL) gave a 5 mmol/L negative bias. Negative bias with 5000 units/mL heparin has been reported previously with methods that use direct ion-selective electrodes (such as the Ektachem) to measure sodium and is presumed to result from binding of sodium by concentrated heparin preparations. Use of more-dilute (1000 units/mL) heparin solution, or flame photometry measurement of sodium (an indirect method as on the Hitachi), showed no negative interference [1].

In routine patients’ plasma diluted to contain 0-300 mL/L of the 5000 units/mL heparin solution, total protein values showed no evidence of interference. However, albumin values from the Hitachi analyzer were falsely increased for samples containing the heparin solution at between 150 and 300 mL/L; i.e., albumin values increased with dilution instead of decreasing. In addition, albumin values were higher than total protein values in routine plasma containing 200-300 mL/L heparin solution, as seen in the dialysis patient. The Hitachi measures albumin by monitoring the interaction between albumin and brom cresol green (BCG), a dye specific for albumin. Both positive and negative interferences from heparin have been reported in the BCG reaction and have been shown to be related to precipitation of the dye complex [2].

To determine whether precipitation of dye complex by heparin and albumin was responsible for the positive bias we observed in Hitachi albumin values, we measured the optical absorbance of Boehringer Mannheim albumin Reagent R1 (containing 115 µmol/L BCG) after the addition of various amounts of albumin and heparin. When 20 g/L bovine serum albumin (BSA; US Biochemical, Cleveland, OH) was diluted to contain 0-300 mL/L of the 5000 units/mL heparin solution and

![Fig. 1. Absorbance spectra of Reagent R1 in the presence of 20 and 40 g/L BSA containing 0, 100, 150, or 300 mL/L heparin solution (5000 units/mL).](image-url)
Detection by $^1$H-NMR Spectroscopy of Chloroquine in Urine from Acutely Poisoned Patient

To the Editor:

High-resolution NMR spectroscopy is increasingly being used to analyze a variety of physiological fluids [1-2] and may constitute a new tool in clinical diagnosis. Many important low-molecular-mass metabolites can be readily detected and quantified by $^1$H-NMR. New sequences, such as $^1$H-$^1$H J-resolved maps, have proved very useful [3]. We have used these methods to investigate a urine sample obtained in a case of acute chloroquine poisoning.

A 41-year-old man was admitted to the hospital after attempting suicide. He was conscious, without neurological deficit; the electrocardiogram showed an increased QT duration, and his blood potassium concentration was 3 mmol/L. A toxicological screen was requested and reported a plasma chloroquine concentration of 890 μg/L.

For NMR measurements, we used a urine sample collected at admission. Spectra were recorded at 300 MHz with a Bruker (Wissenburg, France) AC300 spectrometer. One-dimensional spectra of the crude urine were obtained by operating in the pulsed Fourier-transform mode with quadrature detection; there was a selective reduction in the T2 of the water protons by chemical exchange with NH₄Cl (0.8 mol/L) and a saturation of the water signal. Two-dimensional J-resolved maps of the same urine sample were realized in the same conditions.

We also used a Bruker DMX600 spectrometer operating at 600 MHz with a 5-mm triple-nucleus inverse-geometry self-shielded gradient probe. Two-dimensional total correlation spectroscopy (TOCSY) spectra of urine were obtained with water peak suppression.

For $^1$H-NMR analysis, we added 20 μL of deuterium oxide, containing 3-(trimethylsilyl)-2,2',3,3'-tetradeuteropropionic acid (TMSP-d₄) used as internal chemical shift reference, to 500 μL of the crude urine. The sample was adjusted to pH 7.0 and introduced into a 5-mm-diameter NMR tube; the spectra were run at room temperature.

Compared with that for a normal subject, the $^1$H-NMR spectrum for this patient’s urine shows intense peaks between 1.2 and 1.5 ppm and near 8.3 ppm (Fig. 1A). The J-resolved pulse sequence was used to identify unusual peaks. Expansions of the $^1$H–$^1$H J-resolved map of the sample are shown in Figs. 1B (aliphatic region) and 1C (aromatic region). The spectrum of chloroquine in pure form, run under the same conditions, allowed the assignment of H-2, H-3, H-5, H-12, and H-17 chloroquine protons. The spectrum showed also signals for major endogenous metabolites (e.g., lactate, alanine, 3-d-hydroxybutyrate, hippurate) as well as ethanol.

At 600 MHz, no addition of NH₄Cl (0.8 mol/L) was needed and the water signal was suppressed by a field gradient. The resolution at 600 MHz is much improved over that at 300 MHz, and the one-dimensional spectrum allows us to trace and remove less-intense unusual signals that partly overlap the signals of chloroquine H-2, H-3, H-5, H-12, and H-17 protons, perhaps corresponding to monodesethylchloroquine resonances. The spectrum of monodesethylchloroquine in pure form was run under the same conditions. TOCSY two-dimensional mapping was performed and showed the cross peaks of all the aliphatic and aromatic chloroquine protons. This also showed that the weak unusual peaks near the chloroquine signals on the one-pulse spectrum can be assigned to monodesethylchloroquine protons.

References

In conclusion, the results of high-field $^1$H-NMR spectroscopy at different frequencies (300 and 600 MHz) were coherent and led to the identification of chloroquine in the urine of the intoxicated patient. The major metabolite, monodesethylchloroquine, could be characterized only at the higher field (600 MHz). Because only 500 $\mu$L of biological sample is required, NMR spectroscopy has the potential for use in rapid toxicological screening in routine clinical diagnosis. For this purpose, we will have to characterize by NMR spectroscopy the major xenobiotics involved in poisonings and generate a database of structures and $^1$H-NMR characteristics.

References

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